Section: Miscellaneous

Original Research Article

CLINICOPATHOLOGICAL FEATURES, LABORATORY INVESTIGATIONS AND TREATMENT OF LUPUS NEPHRITIS: A PROSPECTIVE STUDY

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Abstract

Background: Lupus nephritis (LN) is a prevalent complication of systemic lupus erythematosus (SLE) and frequently has a poor outcome due to delay in diagnosis. Histopathological classification of LN along with clinical and laboratory findings can guide us to design a better diagnostic process and treatment plan. The objective is to evaluate clinical profile, pathology, laboratory investigations, and correlate with histopathological classification in patients with LN. **Materials and Methods:** This prospective, open label study was conducted among patients who had SLE with LN. Patients were randomized to receive treatment with National Institute of Health (NIH) and mycophenolate mofetil (MMF) regimens. Demographic and clinical profile, and laboratory findings with histopathological classes were analyzed. **Result:** A total of 52 patients were enrolled with mean age of 27.0 years. Majority of the study population presented with arthralgia (86.5%), edema (80.8%), anemia (63.5%) and renal failure (55.8%). All patients had albuminuria while, 73.1% had hematuria. Majority patients presented in Class IV (40.4%). Out of 45 patients with proliferative LN, 31 exhibited elevated serum creatinine levels and 38 tested positive for double-stranded DNA (dsDNA) antibodies. Majority of class IV patients (70.0% vs. 83.3%) had low Complement component 4 (C4) and Complement component 3 (C3) levels. NIH regimen achieved remission in 22 patients, while in MMF regimen it was seen in seven patients. One death occurred in patients on the NIH regimen. **Conclusion:** Serum creatinine, positive dsDNA along with C3 and C4 levels can be associated with histopathological classes of LN. Nearly similar treatment outcomes were observed in patients on both regimens.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic and potentially fatal disease characterized by a diverse range of clinical symptoms. The disease follows an unpredictable course of organ involvement, and periodic flare-ups can cause long-term damage. Globally, the adjusted prevalence rates of SLE range from 50-100 per 100,000 adults with a significant gender disparity, with approximately 10 women affected for every man.[1] Lupus nephritis (LN) is a common and severe manifestation of the SLE characterized by variable clinical and histological findings and frequently has a poor outcome.[2] The LN affects around 40% of patients with SLE, and approximately 5-30% of them progress to end-stage kidney disease (ESKD), making LN a significant risk factor for mortality and morbidity.[3,4] Patients with LN often exhibit distinct symptoms such as proteinuria, abnormal renal function, and a utilizing renal biopsy as a crucial diagnostic tool.[5]

Measuring Complement component 4 (C4) and Complement component 3 (C3), aids in comprehending the mechanisms and clinically defining LN. As immunological complexes trigger the complement system during disease outbreaks, LN patients usually exhibit low C3 and C4 levels.[6] However, research investigating whether alterations in the plasma concentrations of complement C3 and C4 can function as indicators of a flare-up in SLE has yielded contradictory results.[7,8]

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The treatment plan of LN is guided by the classification of International Society of Nephrology and Renal Pathology Society (ISN/RPS). According to current recommendations, the therapeutic strategy for LN focuses on achieving rapid remission or partial response within 6-12 months, which involves the use of immunosuppressants, adjuvants, and symptomatic drugs.[9,10] Aggressive immunosuppressive treatments, such as mycophenolate mofetil (MMF) and cyclophosphamide (NIH), have shown positive outcomes in patients with proliferative LN (Class III and IV).[11,12]

Based on the identified knowledge gap regarding the use of non-invasive biomarkers such as C3 and C4, the present study aimed to explore the association between various clinical and laboratory findings and histopathological classes of LN, as well as to compare the treatment outcomes.

MATERIALS AND METHODS

Trial Design

This was a prospective, randomized, open-label study was conducted at the SCB Medical College Cuttack between November 2015 and October 2017. This study was conducted in accordance with ethical principles that are consistent with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board/Ethics Committee. Written informed consent was obtained from all the patients prior to study commencement.

Participants

Patients who had SLE with LN diagnosed by history, biochemical and immunological tests and by light microscopy and immunofluorescence study of renal biopsy specimens were included in the study. The patients with SLE without renal involvement and those who were unable to provide informed consent were excluded from this study.

Study investigations and treatment

Necessary blood and urine investigations were conducted for appropriate diagnosis. These included biochemical tests, urinalysis, immunological assays, and viral markers. Confirmatory diagnostic tools used were renal biopsy, light microscopy and immunofluorescence microscopy. The NIH regimen includes intravenous (IV) methylprednisolone (1g/day for 3 days), followed by oral prednisolone (1 mg/kg/day). The cyclophosphamide IV (750 mg/m2-1g/m2) was given monthly for 6 doses, then every 3 months for another 6 doses. Oral steroids were continued and slowly tapered over 6 months. Total leukocyte count and differential count were advised for two weeks after IV cyclophosphamide therapy if any abnormality was found. Whereas, in the MMF regimen, patients received 2 g/day oral dose of mycophenolate mofetil along with prednisolone (1 mg/kg/day), gradually tapering over 6 months then MMF was later replaced with azathioprine.

Outcomes

The objective of this study was to study the clinical and pathological profile, laboratory investigations and treatment outcomes in patients with LN. The secondary objective was to correlate clinical and laboratory findings with histopathological findings of LN.

Randomization: Patients were randomized to 3:1 treatment with NIH and MMF regimen [Figure 1].

Data Collection

The patient's demographic details (age, sex), clinical findings, laboratory reports (urinary protein, urinalysis), serological features, ISN/RPS Classification, serum creatinine, patients with low C3 and C4 levels, and treatment outcomes were recorded on a predesigned, pretested and structured proforma and evaluated.

Statistical Analysis

Statistical testing was done using Statistical Package for the Social Sciences (SPSS) version 21. Descriptive statistics were used to describe categorical variables (frequency and percentages) and continuous variables (mean and standard deviation [SD]).

RESULTS

A total of 52 patients were enrolled in the study with majority (63.5%) belonging to the age group of <30 years. The mean age of the study population was 27.0 years with a female predominance (92.3%). The clinical presentations are represented in table 1. Highest number of patients presented with arthralgia (n=45, 86.5%) followed by edema (n=42, 80.8%), anemia (n=33, 63.5%), renal failure (n=29, 55.8%), malar rash (n=24, 46.2%), hypertension (n=21, 40.4%), oral ulcer (n=20, 38.5%), serositis (n=7, 13.5%), photosensitivity (n=5, 9.6%), alopecia (n=5, 9.6%), and seizure (n=1, 1.9%).

[Table 2] represents various laboratory tests and ISN/RPS classification of study population. The study demonstrated that the mean urine protein level was 3.2 g/day, with all study patients (100%) had albuminuria while 73.1% and 57.7% of patients had hematuria and nephrotic proteinuria, respectively. In following percentages for positive antinuclear antibodies (ANA) [94.2%], double-stranded DNA (dsDNA) [80.8%], anti-Sjogren's-syndrome-related antigen A (anti-SSA) [21.2%], anti-Sjogren's-syndrome-related antigen B (anti-SSB) (17.3%), and anti-smith (11.5%) were observed. The majority of the patients were presented in class IV (40.4%) followed by class III, class III+IV, class IV+V (15.4% each), and class V (11.5%). Of the total patients, 45 (86.5%) have proliferative pathology. The raised serum creatinine was found in 31 (59.6%) patients with proliferative LN. While, positive dsDNA antibodies were found in 38 (73.1%) patients with proliferative LN.

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As depicted in [Table 3], low C3 and C4 levels were seen in ISN/RPS Class IV patients (70.0% and 83.3%, respectively).

Out of 52 patients, 13 patients received MMF and 39 patients received the NIH regimen. The NIH regimen achieved remission in 22 (56.41%) patients, while in the MMF regimen, it was seen in 7 (53.84%) patients. Infection was observed in a slightly lesser number of patients on the MMF regimen (46.15%) than in the NIH regimen (51.28%). Only one death occurred in patients receiving the NIH regimen due to severe infection [Table 4].

Table 1: Demographic characteristics and clinical profile

Parameters	Number of patients (N=52)
Age, years, mean (SD)	27.0 (9.6)
Age groups, years	
<30	33 (63.5)
>30	19 (36.5)
Sex	
Women	48 (92.3)
Men	4 (7.7)
Clinical features	
Arthralgia	45 (86.5)
Edema	42 (80.8)
Anemia	33 (63.5)
Renal failure	29 (55.8)
Malar rash	24 (46.2)
Hypertension	21 (40.4)
Oral ulcer	20 (38.5)
Serositis	7 (13.5)
Photosensitivity	5 (9.6)
Alopecia	5 (9.6)
Seizure	1 (1.9)
Data presented as n (%), unless otherwise specified.	

Table 2: Laboratory investigations and classification of SLE

Investigations	Number of patients (N=52)
Urine protein, (g/day) mean (SD)	3.2 (1.8)
Urinalysis	
Albuminuria	52 (100.0)
Hematuria	38 (73.1)
Nephrotic proteinuria	30 (57.7)
Sub-nephrotic proteinuria	22 (42.3)
Serological features	
ANA	49 (94.2)
Anti-ds DNA	42 (80.8)
Anti-SSA	11 (21.2)
Anti-SSB	9 (17.3)
Anti-Smith	6 (11.5)
ISN/RPS Classification	
Class II	1 (1.9)
Class III	8 (15.4)
Class IV	21 (40.4)
Class V	6 (11.5)
Class III+IV	8 (15.4)
Class IV+V	8 (15.4)
Proliferative LN	45 (86.54)
Serum creatinine, mean (SD) (mg/dL)	2.32 (2.1)
Raised Serum Creatinine in proliferative LN	31 (59.6)
Positive ds DNA with proliferative LN	38 (73.1)
Data presented as n (%), unless otherwise specified. Anti-ds DNA, anti-double-stranded deoxyribonucleic acid; ANA, antinuclear antibodies; anti-SSA, anti-Sjogren's-syndrome-related antigen A; anti- SSB, autoantibodies directed against the SSB; ISN, International Society of Nephrology; LN, lupus nephritis; RPS, renal pathology society.	

Table 3: Complement levels according to ISN/RPS classification

Pathology classification	Low C3 (N=30)	Low C4 (N=24)
Class II	1 (3.3)	-
Class III	6 (20.0)	4 (16.7)
Class IV	21 (70.0)	20 (83.3)
Class V	2 (6.7)	-
Data presented as n (%). C3, complement 3; C4, complement 4.		

Table 4: Treatment outcomes at 6 months

Treatment outcomes	MMF (n=13)	NIH (n=39)
Remission	7 (53.84)	22 (56.41)
Infection	6 (46.15)	20 (51.28)

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Death		1 (2.56)
Data presented as n (%). MMF, mycophenolate mofetil; NIH; National Institute of Health protocol.		

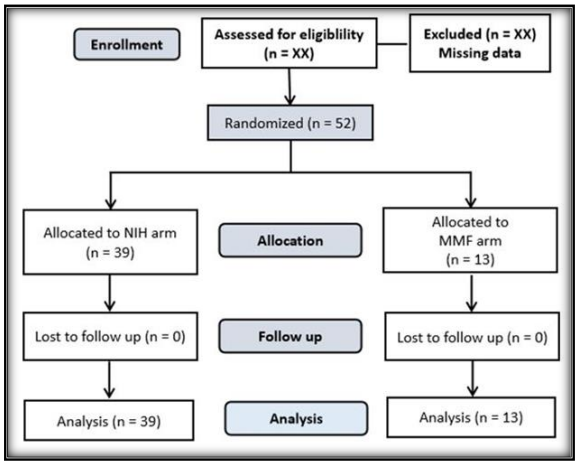

Figure 1: Study flow chart

DISCUSSION

The SLE is a long-lasting, multifaceted autoimmune condition that affects various organs, including the kidney. The intensity of the renal involvement is not usually related to the clinical manifestations of LN, which might range from asymptomatic to having severe nephrotic syndrome or acute nephritis syndrome, making it difficult to confirm the diagnosis. Therefore, in this study, we tried to study the clinicopathological and laboratory findings and the correlation between histopathological classification and the clinical and laboratory findings of the patients with LN. The key findings were i) female predominance; ii) The main clinical features were arthralgia, edema, anemia, renal failure, malar rash, hypertension, and oral ulcer; iii) Serological findings were positive ANA, dsDNA, anti-SSA, anti-SSB and anti-smith antibodies; iv) majority exhibited albuminuria, hematuria and nephrotic proteinuria; v) Majority of the patients belonged to ISN/RPS Class IV; vi) Majority patients with low C3 and C4 levels belonged to ISN/RPS class iv, followed by class III; vii) Majority patients had a proliferative LN with raised serum creatinine and positive dsDNA and viii) The NIH regimen had slightly better treatment outcomes as compared to the MMF regimen.

In various studies, SLE occurred majorly in females,[5,13] which was consistent with the results of the current study (48/52). A retrospective study conducted in the pediatric population revealed that 85.5% of the participants were female.[14] Another noteworthy cross-sectional study conducted on adult patients also noted female sex preponderance (83.3%) in patients with LN.[15]

The most common presenting complaints were arthralgia followed by edema, anemia, renal failure, and malar rash. This is similar to a previous study by Chanda et al., where the presenting problems were arthralgia (82%) followed by a malar rash (70%) and myalgia (66%).[16] Tani et al, who studied the clinical manifestations of SLE, noted arthritis (87.5%), skin rash (50.0%), and mouth ulcers (37.5%) as prominent clinical features in patients with SLE.[17] Another study also revealed similar results indicating pedal edema, arthralgia and malar rash as major clinical features presented within different classes of LN, which were also observed in the present study.[5] The malar rash was found in 46.2% in our patients, consistent with Magal et al.[5]

In a previous study, positive ANA was found in 95.0% of patients, positive anti-dsDNA antibody in 44.1% of patients, positive serum anti-Smith in 10.7%, positive anti-SSA antibody in 64.0% and positive anti-SSB antibody in 22.5% which were in accordance with the present study. In the present it was found that 94.2% tested positive for ANA. Furthermore, 80.8% had positive results for the anti-dsDNA antibody. Among the tested samples, 21.2% tested positive for anti-SSA antibody, 17.4% were positive for anti-SSB antibody, and 11.5% showed positive results for serum anti-Smith antibody.[18] Consequently, patients with SLE exhibited a significant proportion of positive antids DNA antibodies, indicating the presence of an active SLE condition. Therefore, timely diagnosis and proper intervention are crucial.

In parallel to previous studies, this study had majority of the patients with LN belonging to the ISN/RPS class IV.[5,16] Other noteworthy studies determined that Class IV LN was the most frequently discovered class with poor prognosis.[5,19] In the current study, 55.8% of patients presented with renal failure and proliferative LN was observed in 45/52 patients.

Biomarkers such as hematuria, cellular casts, and mild proteinuria were used to diagnose LN.[20] In concordance with the present study, Magal AS et al. found that 23.5% of patients had nephrotic range proteinuria.[5] Lim SC et al. identified 11 clinical factors related to kidney impairment. These factors included nephrotic symptoms, hypertension, C3 and C4 levels, serum albumin and urine protein which were also seen in the present study.[14]

In the present study, patients with SLE with a lower average C3 and C4 levels tended to have a higher risk of subsequent LN (class IV), which is consistent with a previous cross-sectional observational study conducted in 51 patients,[5] demonstrating a strong association between decreased C3 levels and class IV of LN but not with C4 levels. Similarly, A Korean and UK cohort study for patients with SLE reported that a lower C3 level was a risk factor for developing a higher risk of LN.[21,22] Another study by Heidenreich U et al. noted that complement C3 and C4 fractions have extensively different predictions. The C3 and C4 sensitivity was 64.1% and 51.3%, respectively, while specificity was 88.4% and 95.3%, respectively.[23]

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Updated guidelines recommend initiating immunosuppressive therapy for class III/IV or III/IV+V LN with intravenous methylprednisolone followed by a high-dose corticosteroid taper, along with either MMF or cyclophosphamide (NIH) for 3 to 6 months of induction therapy, followed by maintenance therapy using either MMF or azathioprine.[24]

In the present study, patients received the NIH and the MMF regimen for treatment. The treatment outcomes showed that 56.41% of patients on the NIH regimen achieved remission, and infection was observed in 51.28%, while 53.84% of patients on the MMF regimen achieved remission and infection was observed in 46.15% of patients. A randomized controlled trial depicted that MMF was just as efficient as cyclophosphamide at causing remission while causing fewer side effects which aligned with the study.[25] A single event of death was also reported in the NIH regimen of the current study. Therefore, we can conclude that the MMF regimen can be considered over the NIH regimen due to the lesser number of adverse events.

Limitations

The study was conducted in small sample size and a shorter time period making it difficult to extrapolate reliable results.

CONCLUSION

Laboratory investigations like albuminuria, proteinuria and hematuria can be linked with the diagnosis of LN along with serological findings like ANA, dsDNA, and anti-SSA antibodies. Serum creatinine and positive dsDNA, along with C3 and C4 levels, can be associated with histopathological classes of LN. Treatment outcomes with the NIH regimen and MMF regimen were nearly similar, while patients on NIH reported a higher number of infections and there was one patient who died from the NIH group. Therefore, further studies should be conducted in a larger population to extrapolate reliable results.

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